HEPATOCELLULAR TRANSPLANTATION AND TARGETING GENETIC MARKERS TO HEPATIC CELLS

XII. ABSTRACT SUBMITTED WITH RENEWAL APPLICATION TO GENERAL CLINICAL RESEARCH CENTER

Orthotopic liver transplantation represents the only therapeutic option for many children with end stage liver disease or inborn errors of metabolism. Hepatic transplantation in pediatrics continues to be limited by the availability of organs, technical difficulty with transplantation in small children, and the high morbidity, mortality, and expense of the procedure. Two potential alternatives to hepatic transplantation include *hepatocellular transplantation*, in which isolated hepatocytes would be transplanted into the diseased organ, or *somatic gene therapy*, in which the defective function would be replaced in the patient's own cells by gene transfer. *Hepatocellular transplantation* would increase the availability of donor organs, since livers with traumatic damage could be used, and could decrease the morbidity associated with transplantation, since removal of the host organ would not be necessary and immunological rejection may be less severe. *Somatic gene therapy* would be performed with the patient's own hepatocytes isolated by partial hepatectomy, transformed with recombinant genes, and autologously transplanted. Both procedures require development of methods for transplantation and engraftment of isolated hepatocytes.

Animal experiments have demonstrated that *hepatocellular transplantation* will reverse hepatic failure and genetic deficiencies such as analbuminemia and hyperbilirubinemia due to bilirubin, UDP-glucuronyl transferase deficiency (Crigler Najar syndrome). Few of these studies, however, have been able to demonstrate engraftment, since the donor hepatocytes are indistinguishable from those of the host. We have used a recombinant transgene to mark transplanted hepatocytes in mice and demonstrated long-term hepatic engraftment of cells transplanted into the portal vein or spleen. We have also demonstrated efficient gene transfer and expression in primary hepatocytes using retroviral vectors.

We will perform clinical trials of hepatocellular transplantation in six patients with acute, life threatening hepatic failure. Hepatocytes will be harvested from livers that are unsuitable for conventional transplantation and cryopreserved. Subjects will be selected who have been evaluated for orthotopic transplantation; at imminent risk for death or brain damage; have no evidence of irreversible encephalopathy; and no prospect for immediate orthotopic transplantation due to their size (<8-10 kg), availability of suitable organs, or other factors. Active infectious hepatitis, portal hypertension, biliary atresia, or hepatic storage diseases, including alpha$_1$ antitrypsin, would be contraindications. Hepatocytes will be infused into the portal vein, splenic vein, or body of the spleen via laparotomy. In order to assess engraftment, a fraction of the hepatocytes will be cultured and transduced with the LNL6 retroviral vector which carries a NEO-R gene. These transduced cells will be mixed with non-transduced cells prior to transplantation. The NEO-R marker gene can be detected in biopsy or pathological specimens by <u>in situ</u> hybridization, PCR, or enzyme assays and will be used to localize successfully engrafted cells. The LNL6 vector has been used previously in clinical experiments without complications. Subjects will remain listed for orthotopic transplant with UNOS, and conventional transplantation will be performed if the clinical

response is sub-optimal. All experiments will be performed in accordance with NIH guidelines concerning gene transfer into human subjects. This protocol will be used as a model for critical evaluation of biosafety, social, nursing, psychological, and public aspects of *somatic gene therapy*. This protocol is intended to establish clinical and surgical methods for *hepatocellular transplantation* and *somatic gene therapy*.